

THE EFFECT OF DIFFERENT NEUROTRANSMITTERS ON SOME HORMONAL PROFILE OF IMMATURE FEMALE RATS

TAHA, NADIA and ZAKI, A. A.

Physiology Dept. Fac. Vet. Med., Cairo University

SUMMARY

The first objective of the present investigation was to evaluate the relative contributions of the ovarian steroid in the feed back limb of the ovarian pituitary axis. The second was to test the ability of morphine or L-Dopa or both in modulating PRL, PROG, FSH and LH levels noticed in female rats previously treated with estrogen during prepubertal period.

Forty immature female rats were divided into five equal groups and exposed to different treatment. Control olive oil; estrogen; and M. S.; estrogen and L-Dopa; and estrogen, M. S. and L-Dopa. Animals were inspected daily for vaginal opening and estrous manifestation. Blood samples were collected at the end of experimental period and estimated by Radioimmunoassay kits for PRL, LH, FSH and PROG.

The data obtained revealed that sexual precocity elicited either with estrogen or estrogen \pm L-Dopa treatment of the neurotransmitter in hypothalamic sensitivity to estrogen in induction of puberty may be considered.

INTRODUCTION

The establishment of cyclic ovarian activity at puberty is important both for the formation and release of gametes as well as for the establishment

of full sexual development in the female. The reduction in the time taken for puberty by an advance in the age of vaginal opening and first oestrus, are involved in an event of considerable economic commercial importance, particularly in farm livestock.

The advent of female puberty depends on a multiplicity of interrelated events that are initiated within the central nervous system (Ojeda and Urbanski, 1988). This was done by means of hypothalamopituitary system development (Juner et al., 1992). Various neurotransmitters and neuropeptides have been implicated in the control of hypothalamic gonadotrophin releasing hormone secretion (Kalra, 1986). Further, Schanbacher (1985) suggests that opioid modulation of the hypothalamic pituitary ovarian axis involves an interaction with the steroid negative feedback system. Quantitative studies have shown that the physiological steroid level controls LH but does not control FSH (Williams and Lipner, 1982). Also, Urbanski and Ojeda (1986) established that the appearance of LH release during sexual development of the female rat is the consequence of increase in ovarian estradiol secretion. In addition, estrogen administration induces PRL release (Savard et al., 1980) and Wehrenberg et al., (1981). The ways in which steroids and particularly estrogens affect the

release of gonadotrophins are both interesting and contrasting, also less information is available on its possible role.

Based on the aforementioned data, the first objective of the present investigation was designed to evaluate the relative contributions of the ovarian steroid in the feedback limb of the ovarian-pituitary axis. The second was exposure of the hypothalamus and pituitary to sustained elevation of estrogen to elicit a release of gonadotrophins as the preovulatory FSH and LH surge. On the otherhand, this study tests the ability of morphine or L-Dopa or both on modulating PRL., PROG FSH and LH elvels noticed in female rats previously treated with estrogen during the prepuberal period.

MATERIALS AND METHODS

Animals:

The experiment was conducted on forty immature female sprague dawley rats, weighing (70-90 gm) each and housed under a 14 h. light/10h. dark schedule.

They were fed on a complete balanced ration and provided and libitum acces to both fresh water and a trace mineral supplement.

The time of puberty was determined using previously reported criteria (Junier et al., 1992) for control and treated animals that (1) canalization of the vaginal orifice (animals were inspected daily for vaginal opening); (2) sequential manifestation after vaginal opening of the estrous vaginal smears (cornified cells) and

diestrous phases of the first estrojis cycle; and (3) confirmation of the presence of corpora lutea at the time of necropsy.

Study Design:

Animals were randonly divided into five equal groups, eight rats for each, and treated as follows:

(1) Rats were injected intramuscular (i. m.) with 0.2 ml olive oil and served as a control group.

(2) Each rat was i. m. injected with 5 µg estrogen (Folone, Misr Co. for Pharm. Ind.; Mataria, Cairo) in 0.2 ml olive oil/day (Mona A. El-Deen, 1989).

(3) The rats were injected i. m. with 5 µg estrogen in 0.2 ml olive oil/day/rat + morphine sulphate (Misr Co. for Pharm. Ind. Mataria, Cairo) at a dose of 10 mg/kg. b.wt as recommended by Talia (1988).

(4) Rats were injected i. m. with both 5 µg estrogen in 0.2 ml olive oil/day/rat + L. dopa (Mansanto company. St. Louis, Missouri, USA) at a dose of 30 mg/rat/day (Taha, 1992).

(5) Last group treated with i. m. injection of 5µg estrogen in 0.2 ml olive oil/day/rat + morphine sulphate (M. S.) at a dose of 10 mg/kg b. wt/day + L-Dopa at a dose of 30 mg/rat/day.

All previous treatments were conducted for eight successive days.

Blood Collection:

At the end of experiment, blood was taken from the rats by orbital sinus puncture using

heparinized capillary tubes. The blood was allowed to clot for 24 h. at room temperature. Serum was harvested by centrifugation and stored frozen at -20°C until assayed.

Quantification of Hormones:

All serum samples were quantified by radioimmunoassay (RIA) for PRL, LH, FSH and PROG, using kits supplied by Diagnostic Products Corporation 5700 West 96th Street Los Angeles CA 900 45 USA, Serum PRL concentration was quantified according to the method described by Kraeling et al., (1982), assay sensitivity was 1 ng/ml, intrassay and interassay coefficient of variation were 16.3% and 15.2% respectively.

Serum concentrations of LH and FSH were quantified by double-antibody RIAs (Guthrie and Bolt 1983), for LH the intra-and inter-assay coefficients of variation (CVs) were 11.1 and 16.4% respectively and sensitivity was 35 pg/tube, for FSH the intra-and inter-assay CVs were 6.1 and 20.2% respectively and sensitivity was 50 pg/tube. Concentrations of PROG were determined in the one assay (Coleman et al., 1984), the intra-assay CV was 4.3% and sensitivity was 10 pg/tube.

Statistical Analysis:

Hormonal concentrations were reported as a mean+ standard error. Analysis of data was done using "F" test according to Snedecor (1956)

A data set consisting of FSH, LH, PRL and PROG levels within each rat created to characterize the

diversity of these hormone values within animals during the prepubertal maturation treated with estrogen alone or with some neurotransmitters (Table 1).

RESULTS

Regarding gonadotrophins, FSH (Fig. 3) decreased in two groups (estrogen and estrogen +M.S.), while increased in estrogen + L-Dopa treated group. Fig (4), depicts parameters of LH levels, there was a significant increase in groups treated with estrogen and estrogen plus L-Dopa and decrease LH level following estrogen + M. S. administration. No effect was detected in LH from treatment with estrogen + M. S. + L-Dopa.

The hormonal changes detected in treated animals revealed that, PRL levels (Fig. 1) significantly increased in estrogen and estrogen plus M. S. treated groups, while decreased in group treated with estrogen plus L-Dopa and group administered estrogen plus M. S. L-Dopes compared to its control.

Mean serum PROG concentrations (Fig. 2) show that, its significantly increased in three treated group (estrogen + M. S., estrogen + L-Dopa and estrogen + M. S. L-Dopa). however no change in estrogen treated group comparing to its control.

Sexual precocity elicited by treatment with both estrogen and estrogen plus Dopa administration (29.1 ± 0.1 days) as compared to control untreated rats (34.0 ± 0.2 days) otherwise there is no change in other treated group.

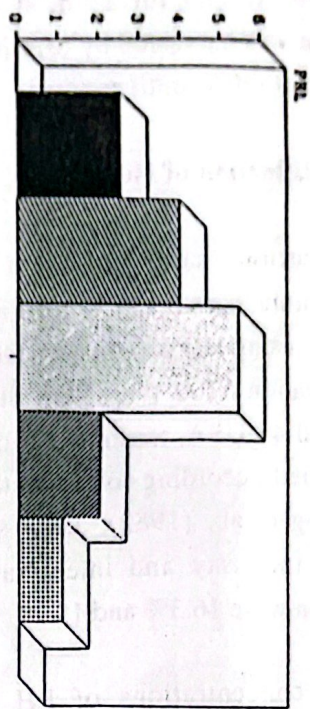


Fig. (1): Effect of different treatments on serum PRL levels

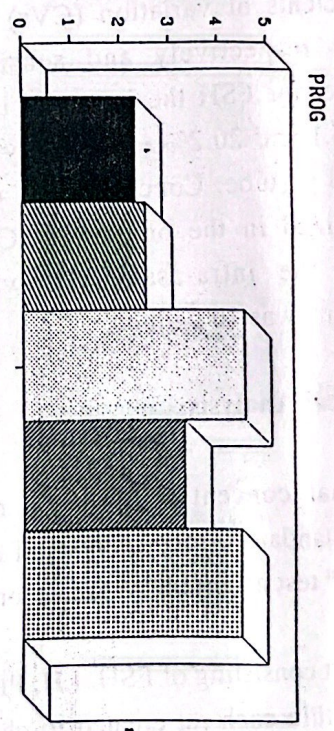


Fig. (2): Effect of different treatments on serum PROG levels

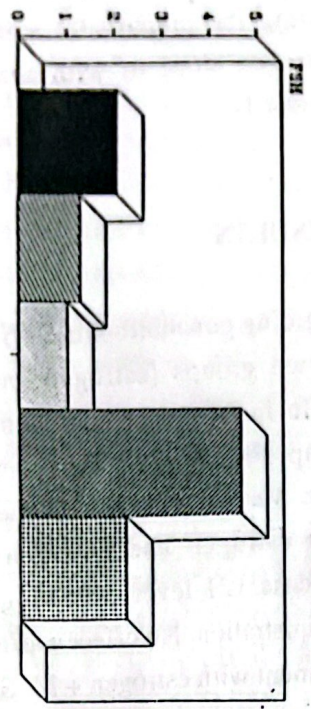


Fig. (3): Effect of different treatments on serum FSH levels

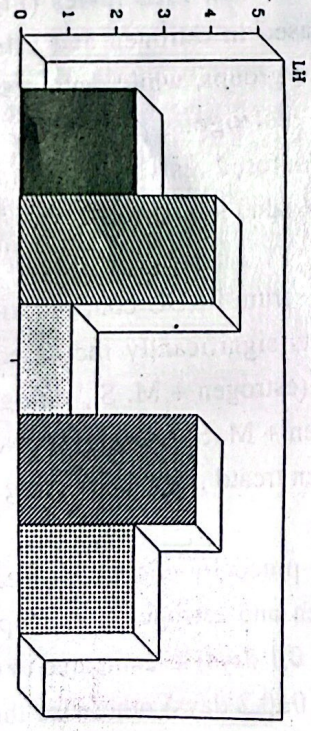


Fig. (4): Effect of different treatments on serum LH levels

Table (1): Effect of different treatments on serum FSH, LH, PRL and PROG levels.

Hormonal levels Groups	FSH (m.I.µ-/ml)	LH (m.I.µ-/ml)	PRL (m.I.µ-/ml)	PROG (m.I.µ-/ml)
Control group (olive oil)	2.08	2.42	2.58	2.34
Estrogen (0.50 µg)	1.32	4.07	3.98	2.53
Estrogen + Morphine sulphaf (10 mg/kg.b.wt.)	1.03	1.08	5.41	4.54
Estrogen + L-Dopa (30 mg/rat)	4.66	3.71	2.03	3.29
Estrogen + Morphine sulphate + L-Dopa	2.23	2.41	1.10	4.43
L.S.D. at 5 %	0.40	0.55	1.10	1.03
F	105.80	40.05	19.95	8.40

DISCUSSION

Several aspects of follicular development during prepuberty remain unclear, classically FSH act in a coordinated fashion to control follicular development and to initiate the morphological and functional differentiation of granulosa cells into luteal cells (Ainsworth et al., 1990). Secretion of LH from the pituitary is primarily under the control of GnRH (King et al., 1989). This paper describes changes in the serum LH and FSH concentration in immature rats, the results show that, the level of FSH significantly increased with estrogen + L-Dopa injection significantly elevate serum LH level. On the other hand LH decrease in group treated with estrogen + MS, this result raises the possibility of estrogen enhancement of stimulated LH release (Ramey et al., 1987) appear to be due to its action or synthesis or release of LH (Liu and Jackson, 1981). Morphine inhibits LH release by increasing hypothalamic

serotonergic activity (Leiri et al., 1980).

Concerning PRL level, the data suggest that a significant increase in PRL following estrogen and estrogen +M. S. administration and decrease in estrogen + L-Dopa and estrogen + M. S. L-Dopa groups. The main controlling factor for PRL is inhibitory, the PRL inhibiting factor is thought to the dopamine (Enjalbert et al., 1979). Estrogen directly stimulates PRL synthesis (Betheat and Yuzuriha, 1986). Thus estrogen effect in PRL regulation may be derived from the ability of estrogen to increase PRL secretion in the absence of hypothalamic inhibition (Frawley and Neill, 1980) or to overcome hypothalamic inhibition at either the pituitary or a neural level (Bethea the Yuzuriha, 1986). It now appears that results are consistent with the known physiology of PRL regulation.

The data presented here revealed that, PROG increased significantly within three different treatments (estrogen + M. S. estrogen + L-Dopa and estrogen + M. S. + L-Dopa) groups, while estrogen alone fails to modulate PROG level in prepubertal animals. In agreement with our result, Sanchez-Criado et al., (1986) stated that, PROG concentrations were higher for animals exhibiting an LH surge.

There are basically two ways by which PROG secretion be controlled. One is PROG itself which may inhibit estradiol secretion acting on follicular growth and follicular steroidogenesis directly through granulosa cell PROG receptors (Schreiber and Ericson, 1979) and / or indirectly through the hypothalamic-pituitary axis especially via lowering serum LH levels (Taya et al., 1981).

In conclusion, sexual precocity elicited either with estrogen or estrogen plus L-Dopa treatment. The time of vaginal opening was used as a criterion for determining the onset of puberty. In accordance with our finding. Ojeda et al., (1976) has shown that the first ovulation in the rat is preceded by a proestrous like increase in gonadotrophin from the adenohipophysis, which is believed to be responsible for triggering the first ovulation at puberty (Advis et al., 1978). However, the possible involvement of some neurotransmitter as L-Dopa in hypothalamic sensitivity to estrogen in induction of puberty may be considered.

REFERENCES

- Advis J. P., Simpkins J. W., Chen H. T. and Meites J. (1978): Relation of biogenic amines to onset of puberty in the female rat. *Endocrinology* 103, No. 1: 11-16.
- Ainsworth L., Tsang B. K., Downey BR. and Maccus G. J. (1990): The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. *J.Reprod Fertil (Suppl)* 40: 137-150.
- Bethga C. L. and Yuzuriha E. (1986): The effect of estrogens and progestins on monkey prolactin synthesis. *Endocrinology* vol. 119 No. 2: 771-779.
- Coleman D. A., Flemin M. W. and Dailey R. A. (1984): Factors affecting ovarian compensation after unilateral ovariectomy in goats. *J. Anim. Sci.* 59: 170-176.
- Enjalbert A., Ruberg M., Arancibia S., Priam M. and Kordon C. (1979): Endogenous opiates block dopamine inhibition of prolactin secretion in vitro. *Nature* 290, 590, 597.
- Frawley L. S. and Neill J. D. (1980): Effect of estrogen on serum prolactin levels in rhesus monkeys after hypophyseal stalk-transection. *Biol. Reprod.* 22: 1089.
- Guthrie HD and Bolt DJ (1983): Changes in plasma estrogen, luteinizing hormone, follicle-stimulating hormone and 13,14-dihydro-15-keto-prostaglandin F₂ during blockade of luteolysis in pigs after human chorionic gonadotropin treatment. *J. Anim. Sci.* 57: 993-1000.
- Ieiri T., Chen H. T. and Meites J. (1980): Naloxone stimulation of luteinizing hormone release in prepubertal female rats; role of serotonergic system. *Life science*, Vol. 26, 1269-1274.
- Junier M. P. Wolff A., Hoffman G. E., Ying Jun M. A. Ojeda S. R. (1992): Effect of hypothalamic lesions that induce precocious puberty on the morphological and functional maturation of the luteinizing hormone-releasing hormone neural system. *Endocrinology*. 131 (2) 787-798.
- Kalra, S. P. (1986): Neural circuitry involved in control of LHRH secretion: a model for preovulatory LH release. *Frontiers in Neuroendocrinology* 44, 31-75.
- King J. A., Davidson J. S. and Mehl a E. I. (1989): Gonadal steroid modulation of signal transduction and luteinizing hormone release in cultured chicken pituitary cells. *Endocrinology*. Vol. 124: 1830-1840.
- Kraeling R. R., Rampacck G. B., Cox N. M. and Kiser T. E. (1982): Prolactin and luteinizing hormone secretion after bromocriptin (CB-154) treatment in lactating sows and ovariectomized gilts. *J. Anim. Sci.*, 54, 1212-1220.
- Liu T. C. and Jackson G. L. (1981): Big luteinizing hormone: possible precursor of native LH in anterior pituitary glands of rats. *Biol. Reprod.* 24: 380.

- Mona, M. H. Ala El-Deen (1989): Reconstitution of idiotypes of bovine prolactin. M. V. Sc. Thesis, Faculty of Vet. Med. Cairo University.
- Gijeda S. E., Wheaton J. E., Jameson H. E. and McCann S. M. (1976): The onset of puberty in the female rat changes in prolactin, gonadotropins, luteinizing hormone-releasing hormone (LHRH) and hypothalamic LHRH content. *Endocrinology*. 98: 630.
- Ojeda S. R. and Urbanski H. F. (1988): Puberty in the rat. In the physiology of reproduction, pp. 1699-1738. Ed E. Knobil and J. Neill. New York. Raven press.
- Ramey J. W., Highsmith R. F., Wilfinger W. W. and Baldwin D. M. (1987): The effect of gonadotropin-releasing hormone and estradiol on luteinizing hormone biosynthesis in cultured rat anterior pituitary cells. *Endocrinology*. 120, No. 4: 1503-1513.
- Sanchez-criado J. E., Lopez F. and Aguilar E. (1986): Pituitary regulation of corpus luteum progesterone secretion in cyclic rats. *Endocrinology*. 119 No. 3: 1083-1088.
- Savard P., Barden N., Merand Y., Rouleau D. and Dupont, A. (1980): Effect of estrogen and haloperidol treatment on brain met-enkephalin levels and receptors in rats. 63 *Endocrine Soc.*, Abstract No. 586, P. 221.
- Schanbacher B. D. (1985): Endogenous opiates and the hypothalamic pituitary gonadal axis in male sheep. *Dom. Anim. Endo.* Vol. 2 (2): 67-75.
- Schreiber JR. and Ericson JF. (1979): Progesterone receptor in the rat ovary: further characterization and location in the granulosa cell. *Steroids* 34:459.
- Snedecor, G. W. (1956): *Statistical Methods*. 5th Ed. Ames. Iowa State Univ. Press.
- Taha, N. A. (1988): Some physiological studies on the prolactin hormone in male rats. Ph. D. Thesis Fac. Vet. Med. Cairo University.
- Taha, N. A. (1992): Effects of some dopamine precursors and against on some serum electrolytes and enzymes in young and old male rats. *J. Egypt. Vet. Med. Ass.* 52, No. 2, 169-182.
- Taya K., Terranova PF. and Greenwald G. S. (1981): Acute effects of exogenous progesterone on follicular steroidogenesis in the cyclic rat. *Endocrinology* 108: 2324.
- Urbanski H. F. and Ojeda S. R. (1986): The development of afternoon minisurges of luteinizing hormone secretion in prepubertal female rats in ovary dependent. *Endocrinology*. Vol. 118, 2: 1187-1193.
- Wehrenberg W. B., Wardlaw S. L., Frantz A. G. and ferin M. (1981): B-endorphin in hypophyseal portal blood in primates: variations throughout the menstrual cycle. 63 *Endocrine Soc.*, Abstract No., 47, p. 94.
- Williams A. T. and Lipner H. (1982): The contribution of gonadostatin (Inhibin-F) to the control of gonadotropin secretion in a stimulated estrous cycle in steroid treated ovariectomized rats. *Endocrinology*. Vol III (1): 231-237.